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ENDOGENOUS OXYGEN UPTAKE AND SPECIFICITY OF EMBRYONIC INTRACELLULAR CONSTITUENTS [1]

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The action and interaction of the various internal parts of the living cell, especially as they relate to its normal functioning, have long been of interest to the experimental biologist. That the reactions of living cells are the results of enzyme-substrate relationships seems well established but many of the details of these phenomena are as yet not clear. The extent to which the nucleus controls or regulates the type or nature of chemical reactions occurring in the cell as a whole has to a great degree been discussed from a theoretical rather than from a scientific point of view (DeRobertis, Nowinski and Saez, 1950). The presence, distribution and localization of intracellular enzyme-substrates and chemical compounds have been variously demonstrated by micro-chemical and other techniques (Caspersson, 1950). The fact that living cells are in themselves able to carry on the reactions essential for their metabolic activities also seems well established. The degree to which the different parts of the cell are specific for these physico-chemical phenomena is at present not too well understood (Caspersson, 1950; Brachet, 1950). Details as to the presence of typical chemical compounds in the different parts of living cells are rapidly accumulating and tend to show a marked regional localization for many of the materials deemed essential for the various functions of the parts of the cell (Lardy, 1949; Caspersson, 1950). Results from many investigations on the enzyme-substrate relations of cellular parts seem to deal largely with systems isolated from the cells and to which various reagents have been added and, conclusions then deduced from results of such extracellular experiments, are referred to the possible conditions in the intact normal cell (Schneider, 1946; Schneider, Claude and Hogeboom, 1948; Lardy, 1949). Valuable data, however, have accumulated from investigations carried out by these methods. It seems reasonable to assume that investigations of the endogenous metabolism of the intact living cell and its parts should contribute additional data to an understanding of intracellular phenomena. To this end, extensive experiments have been carried out on the endogenous oxygen uptake of the various parts of the normal living embryonic cells of the grasshopper embryo. The embryo of the grasshopper (*Melanoplus differentialis*) has many advantages for such investigations since it can be rigidly controlled and standardized (Bodine and Lu, 1950a). Yolk and other extraneous materials

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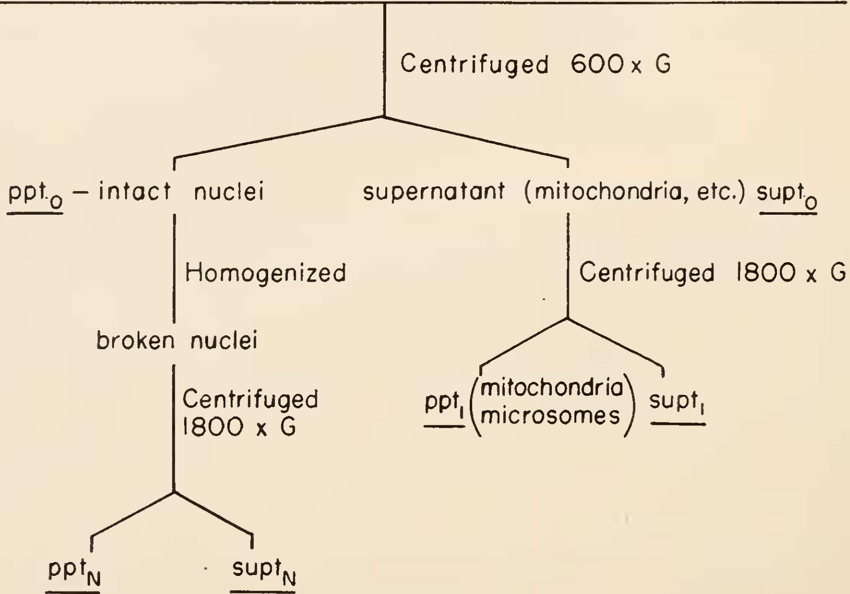
which normally interfere to a marked degree in such experimental work can be practically eliminated. Since the embryo is a cold-blooded animal, rigid temperature and developmental controls can easily be maintained (Slifer, 1931). The present data are concerned with the endogenous oxygen uptake of the homogenates and intracellular parts of the embryonic cells of mitotically active (postdiapause) and developing embryos.



$supt_O$ = supernatant from centrifugation of homogenate of embryo – cytoplasm (microsomes, mitochondria, substrate)
ppt_O = precipitate " " " " " " – nuclei (intact).
ppt_N = " " " " " " nuclei – solid constituents of nuclei.
$supt_N$ = supernatant " " " " " " – non-solid constituents of nuclei
ppt_I = precipitate " " " " " cytoplasm – mitochondria, microsomes(?)
$supt_I$ = supernatant " " " " " " – largely substrate (?)

FIGURE 1.

MATERIALS AND METHODS

Embryos of the grasshopper (*Melanoplus differentialis*), dissected from eggs of known developmental and temperature history, have been used throughout, while all methods employed were essentially similar to those already described (Bodine and Lu, 1950a). Oxygen uptake was measured with standard Warburg manometers at 25° C. using flasks of 5 ml. capacity. Micro-differential manometers were used for isolated nuclei and nuclear homogenates. Preparation of homogenates and

fractionation of cellular parts were carried out as previously indicated (see Fig. 1) (Bodine and Lu, 1950a). Cytological examinations, using the phase microscope, were made on all materials used. Ringer's solution, phosphate buffered (pH 6.8), was used as suspension medium. Concentrations of intracellular constituents were so chosen that 1 ml. of medium contained the equivalent of those obtained from 100 embryos of known developmental history. Figure 1 gives a diagramatic outline of procedures as well as symbols for fractions produced.

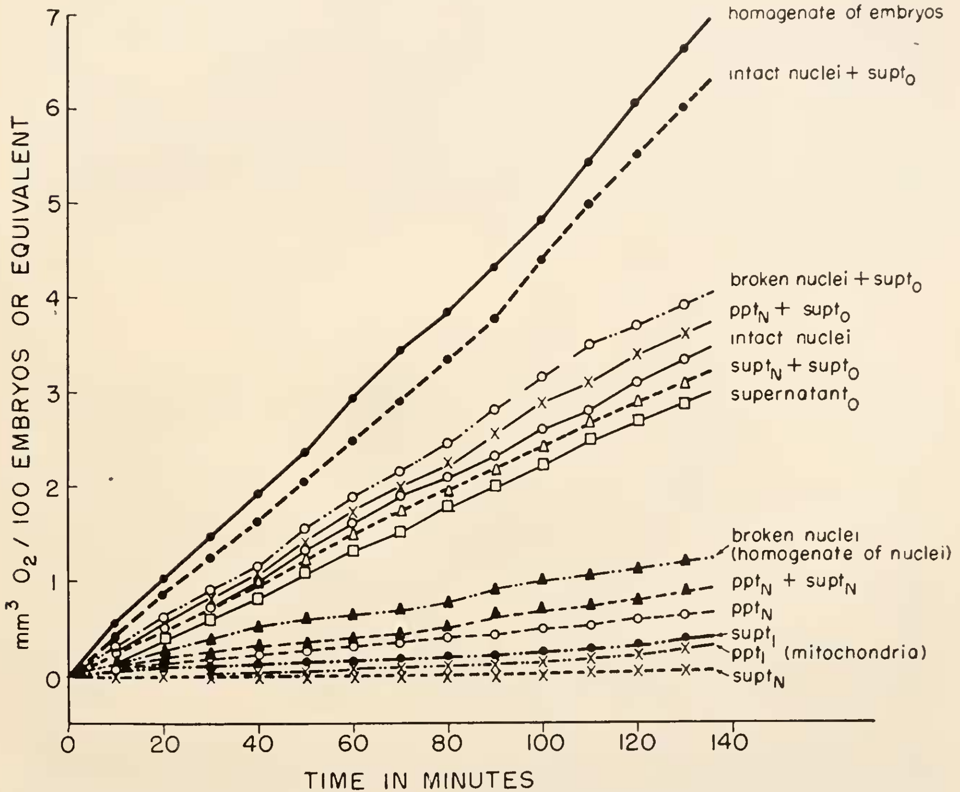


FIGURE 2. Shows oxygen uptake of homogenate of embryo and its constituent parts. Ordinate, mm.^{3} oxygen per 100 embryos or its equivalent. Abscissa, time in minutes. Symbols same as in Figure 1.

RESULTS OF EXPERIMENTS

Inasmuch as results for different lots of embryos are qualitatively similar, only typical cases for eggs of uniform ages and developmental histories will be presented. In general, all embryos and their products thus far tested have given similar results.

The relations between the oxygen uptake of mitotically active (postdiapause) and blocked (diapause) embryos and their homogenates have previously been pointed out (Bodine, 1950). We shall deal here with the endogenous oxygen uptake of embryo homogenates, nuclei, and nuclear homogenates, as well as specific

differences and similarities between enzyme-substrates in the various intracellular parts. Results for typical experiments are graphically shown in Figures 2 and 3.

An examination of Figure 2 shows that the endogenous oxygen uptake of fractionally separated intact nuclei and supernatant (cytoplasm) is approximately 50 per cent of that for the homogenate from which they are derived (Bodine and Lu, 1950a). Upon recombination of the two parts ($ppt._o$ + $Supt._o$) oxygen uptake is approximately 90–95 per cent of that for the original homogenate. These results confirm those previously reported for somewhat similar experiments (Bodine and Lu, 1950a). The intact nuclei apparently have their own enzyme-substrate systems which seem quite distinct from those of the cytoplasm. The cytoplasm, on the other hand, contains both enzymes (mitochondria, microsomes) and substrates (Bodine and Lu, 1950b). Washed and concentrated mitochondria and microsomes respire little if at all and the same is true for the cytoplasmic supernatant after their removal from it (Figs. 2 and 3). Since washed intact nuclei can be broken by homogenization (Bodine and Lu, 1950b), it becomes of some interest to compare

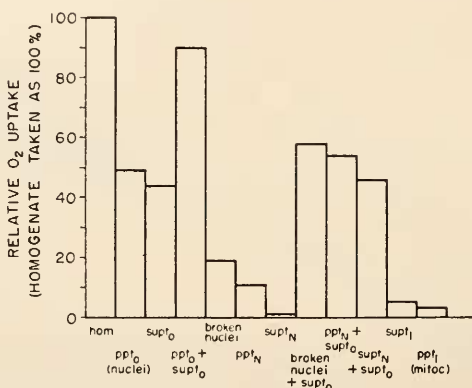


FIGURE 3. Shows relative oxygen uptake for 120 minutes in terms of homogenate of embryo as 100 per cent. Symbols as in Figure 1.

oxygen uptake of intact nuclei with that of their constituent parts and also to determine, if possible, something of the relative specificity of enzyme-substrates of nuclei and cytoplasm. Oxygen uptake of broken nuclei (homogenate) is reduced below that of the intact ones and falls to approximately 30–35 per cent of the original value (Figs. 2 and 3). The broken nuclei are readily separated by fractional centrifugation into solid particles and a liquid supernatant. The oxygen uptake of the supernatant nuclear fluid is practically zero while the solid fraction, even though washed, respires at approximately 50 per cent of the nuclear homogenate rate (Figs. 2 and 3). By recombining the nuclear fragments with the fluid supernatant, a close approximation to the original oxygen uptake is obtained, similar to the case of recombinations of the constituent parts of embryonic homogenate (Bodine and Lu, 1950b).

By making suitable combinations of nuclear and cytoplasmic fractions, rather striking evidence for a marked degree of specificity in the endogenous oxygen enzyme-substrate systems becomes apparent. Broken nuclei (or homogenates of

nuclei) added to cytoplasm produce a greater oxygen uptake than cytoplasmic or nuclear homogenates alone and the sum total of the oxygen uptake is approximately equal to the sum of that for the two systems (Fig. 3). Nuclear supernatant added to cytoplasm produces no significant change in the oxygen uptake over that for cytoplasm alone. The solid particles of the nuclei added to cytoplasm produce an oxygen uptake equal to that of the sum of the two constituent fractions. It seems reasonable to assume, therefore, that the enzyme activity of the nucleus is confined to the solid parts and that substrate resides largely in the fluid parts. Since cytoplasm produces no marked increases in oxygen uptake when added to nuclear fluids, it would seem that mitochondria and other enzyme-bearing structures in cytoplasm do not act upon nuclear substrates and that a degree of specificity exists for cytoplasm and nucleus as regards this type of reaction (Figs. 2 and 3). Inasmuch as endogenous oxygen consumption is that confined *entirely* to the inherent enzyme-substrate systems of the cell, it is not possible from the above results to separate or point out specific types of enzymes involved in these reactions, but it does seem reasonable to assume that some degree of specificity does exist for the nuclear and cytoplasmic respiration systems.

Discussion

The extent to which a specificity for the enzyme-substrate systems of the cytoplasm and nucleus can be demonstrated should throw some light upon the probable chemical interchanges between these structures in the normal intact cell. Evidence gained from the localization of certain chemical compounds like the nucleic acid derivatives would seem to indicate but little passage of materials between the two parts (Caspersson, 1950). That some substances must constantly interchange between the two structures seems reasonable to suppose in view of the equilibrium normally existing between them (Caspersson, 1950; DeRobertis, Nowinski and Saez, 1950). From the present observations on the embryonic cells of the grasshopper, it would seem that for the endogenous oxygen uptake studied, the two systems are more or less distinct. Such a postulation would hold only for those enzyme-substrate systems connected with the endogenous oxygen uptake and would in no way contribute information as to the parts played by other systems. Since the living cell of the grasshopper embryo is stimulated by 2,4-dinitrophenol only when *intact,* while methylene blue stimulates oxygen uptake almost solely by its action on the intact nucleus, one might reasonably assume from such reactions a marked difference in the physico-chemical workings of the various parts of the cell (Bodine and Lu, 1950c). Evidence recently acquired shows that succinate added to homogenates of embryo increases oxygen uptake while no effect is noted when intact or homogenized nuclei are employed. Structural or morphological relations and "intactness" of cells undoubtedly contribute much to the rates at which various localized enzyme-substrate systems work. Localization of substrates in the intact cell is greatly changed or disturbed in homogenates. However, in the case of the embryo, isolated intact nuclei—when combined with cytoplasm—show oxygen uptake of approximately 90–95 per cent of that found for the homogenate from which the constituents were taken (Bodine and Lu, 1950b).

Summary

1. The endogenous oxygen uptake of the homogenate of the embryo of the grasshopper, *Melanoplus differentialis,* along with the intracellular constituents of the embryonic cells has been measured.

2. The endogenous oxygen uptake of intact washed nuclei and their homogenate as well as that of the intranuclear constituents has also been measured.

3. Respiratory enzymes of the nucleus are located in the solid particles obtained from fractional centrifugation.

4. Enzymes of cytoplasm and nucleus appear specific since no change in oxygen uptake occurs when they are added to reciprocal substrates.

5. Fractions of homogenates of embryo or nuclei when recombined have oxygen uptakes of approximately 90–95 per cent of the original homogenates.

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